

CONTROLLING SOLUBILITY, HALF-LIFE, TOXICITY AND TARGETING

The use of macromolecule conjugates to enhance the performance of pharmaceuticals is currently an area of intense research for both small molecules and biological therapeutics. Starpharma has been applying its dendrimer technology to address important challenges in drug development, an approach which is now gaining favour in the industry.

Developing a new drug is a complex task in which many different parameters must be optimized, including potency, toxicity, bioavailability, biodistribution, clearance rates, formulability and defensible patent position.

There are a limited number of variables available to address all of these constraints in a single molecule. For this reason conjugating drugs to macromolecular “vehicles” is increasingly being considered as a means of breaking the problem into manageable pieces. For example, one may choose to optimise potency in the structure of the drug itself, but leave control of biodistribution to a vehicle such as a conjugated macromolecule. In this note we will describe how Starpharma’s dendrimer-conjugate technology allows this division of labour between drug and vehicle to be achieved in practice.

FIVE KEY APPLICATIONS

Starpharma has focused on five key drug delivery objectives:

Increase Solubility – by conjugating the drug to a dendrimer construct, very large increases in drug solubility have been achieved.

Control Half-Life – the dendrimer can protect drugs from degradation and inhibit renal clearance. The half-life of drugs have thereby been substantially increased.

Control Off-Target Toxicity – different dendrimers can keep drug away from different tissues. This has been used to reduce dose-limiting toxicities.

Target Organ, Tissue or Receptor – dendrimers have been used to actively or passively target a payload drug to particular destinations.

Improve Efficacy - dendrimers can enable drugs to be delivered over a longer timeframe and target diseased tissues resulting in enhanced efficacy.

WELL-DEFINED, VERSATILE, SYNTHETIC MACROMOLECULES

Starpharma’s dendrimers are highly-branched macromolecules with a well-defined structure. Starting with a core molecule, branching lysine units are repeatedly added in layers (or “generations”), until the desired structure is reached (figure 1).

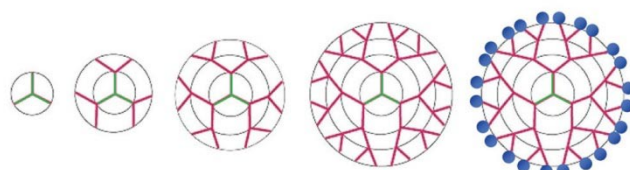


Figure 1: A dendrimer is a macromolecule with a well-defined highly-branched, 3D structure. It is synthesised in spherical layers, by adding monomers onto a core. For most of Starpharma’s pharmaceutical dendrimers the monomer of choice is lysine, which is biofriendly and low-cost.

The many surface points of attachment can then be functionalised with one or more groups depending on the intended application (figure 2).

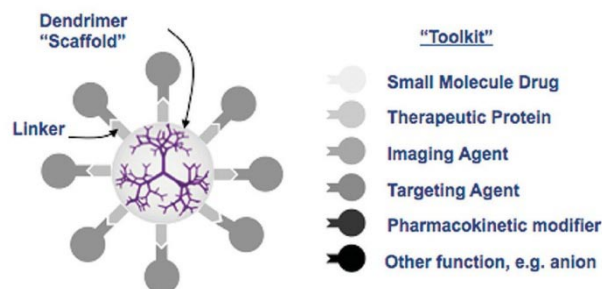


Figure 2: The surface of the dendrimer can be modified to achieve a wide range of functionality. Typically one or more functional groups are used on 32 to 64 attachment sites.

Starpharma has taken its polylysine dendrimers into the clinic in the form of a microbicial vaginal gel, VivaGel®. VivaGel® is under Phase 3 clinical development for the treatment and prevention of bacterial vaginosis (BV) and also as a vaginal microbicide to prevent the transmission of sexually transmitted infections including HIV and genital herpes. VivaGel® is the subject of separate licensing agreements with Ansell and Okamoto, to market a value-added, VivaGel®-coated condom. Ansell manufactures and sells leading condom brands worldwide and Okamoto is the market leader for condoms sold in Japan, the world’s second largest condom market.

VivaGel® is manufactured under cGMP and its structure is closely related to many of the dendrimers that Starpharma uses for drug delivery applications.

A growing number of pharmaceutical companies are now working with Starpharma: the company has collaborations with Lilly, GSK’s Stiefel Laboratories, and a number of additional undisclosed partners.

Below we give some examples of well-known drugs that we have modified with dendrimers to achieve different objectives.

INCREASING SOLUBILITY

Paclitaxel is a cancer drug well known for its poor aqueous solubility (<1µg/ml). When conjugated to a dendrimer construct a 9000 fold increase in solubilised paclitaxel is achieved (figure 3)



Paclitaxel, no dendrimer
Solubility 0.8 µg/ml

Paclitaxel with dendrimer
Solubility >37 mg/ml

Figure 3: By conjugating paclitaxel to a dendrimer construct its aqueous solubility is enhanced 9000-fold.

The structure is designed to release the active pharmaceutical ingredient (API) in the body so that it can achieve its intended pharmacological effects. Similar impressive results have recently been achieved with the leading chemotherapy drug docetaxel, a closely related cancer drug with blockbuster annual sales.

(See Inset Box "Linkers – controlling where the drug is released").

CONTROL OF HALF-LIFE

The half-life of a drug can be substantially enhanced by attaching it to a dendrimer construct. For example attaching methotrexate (MTX) to different members of a family of dendrimer constructs leads to a range of clearance rates in rats (figure 4), ranging from 24 minutes (free MTX), to nearly 24 hours shown in this figure, and 50 hours in a related construct.

A comparable result was achieved with doxorubicin (DOX): a half-life of approximately 30 minutes for free DOX was extended to 34 hours for a DOX-dendrimer construct.

Linkers: Controlling where the drug is released

Some drugs continue to function whilst attached to dendrimers, whereas others need to be released before they can work. Starpharma uses different kinds of linker between the dendrimer and the drug to control when and where drug is released, for example:

"Permanent" Linker

If the drug works satisfactorily when attached to the dendrimer, then there may be no need to release it, as in the case of insulin.

Hydrolytically Unstable Linker

Sometimes sustained release of the active may be all that is required, with no preferred release location. In this case a linker that breaks down with a certain half-life in aqueous solution may be the correct choice.

Acid-Labile Linker

Newly forming tumors are often hypoxic, and therefore have a low pH. By using a linker that is stable at neutral pH, but unstable at lower pH it is possible to preferentially release drug at a tumour.

Enzymatically Cleaved Linker

It is possible to preferentially release a drug molecule at the location where a specific enzyme occurs by connecting the drug to the dendrimer with a linker that is subject to enzymatic degradation. Such locations include tumours where certain enzymes are commonly over-expressed.

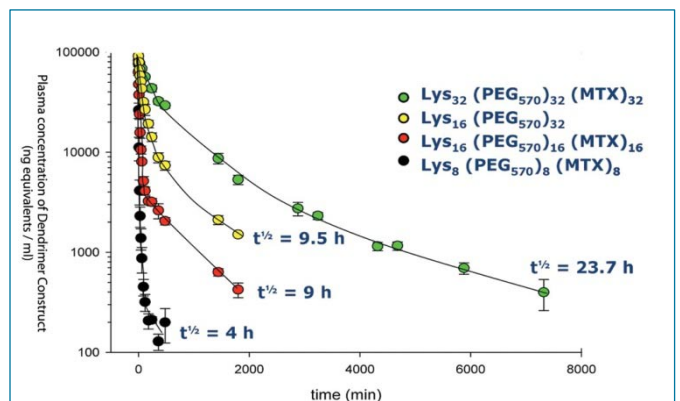


Figure 4: Attaching small molecule drugs to dendrimer constructs allows control of drug half-life. In this study the half-life of methotrexate (MTX) was extended from 24 minutes to 24 hours. For a different methotrexate construct a 50 hour half-life was achieved.

The approach is applicable both to small molecules and biological therapeutics. In *figure 5* the activity of insulin is prolonged so that glucose levels are suppressed longer in mice receiving insulin-dendrimer construct compared with mice receiving insulin alone.

In the case of doxorubicin and methotrexate it was necessary for the API to be released from the construct to function. For insulin, release was not required. (See *Inset Box "Linkers – controlling where the drug is released"*).

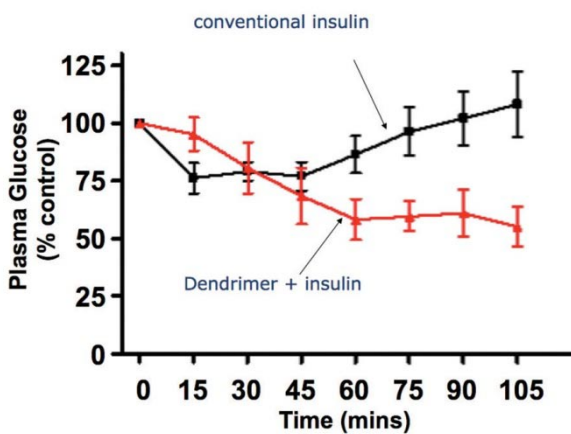


Figure 5: The half-life of biological molecules can also be extended using dendrimers. In this case the effective duration of activity of insulin is seen to be prolonged in mice compared to insulin alone

CONTROL OF TOXICITY

Damage to heart muscle can be a dose limiting toxicity for the cancer drug doxorubicin (*figure 6a*). However, when doxorubicin is attached to a suitable dendrimer construct with preferential release of drug near a tumor (See *Inset Box "Linkers: controlling where the drug is released"*), much less free drug reaches the heart, and cardiac damage is reduced, (*figure 6c*), whilst drug efficacy is maintained at the tumour site (*figure 6d*).

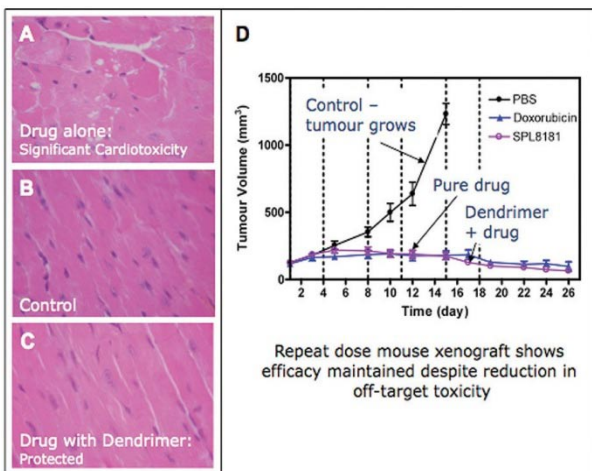


Figure 6: (A) Cardiac tissue damage can be a dose limiting toxicity for doxorubicin. (B) Image of undamaged cardiac muscle where negative control (PBS) is administered. (C) When DOX is conjugated to dendrimer, heart tissue no longer becomes damaged despite retention of efficacy.

TARGETING

There are two mechanisms that may be advantageously employed to control the destination of drugs when attached to a dendrimer.

Active Targeting – Adding a suitable targeting molecule to the construct, such as an antibody, allows the dendrimer to carry a payload to a target receptor. In *figure 7* this is achieved for a payload of gadolinium, allowing visualization in an MRI scanner. However the payload could equally have been a small molecule API.

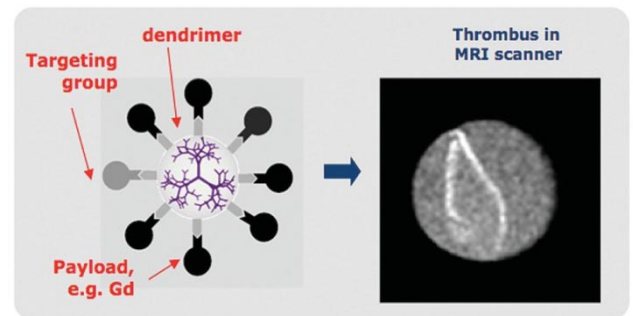


Figure 7: Left: By associating a dendrimer with a targeting group (e.g. an antibody) multiple "payload" molecules can be delivered to a molecular target. Right: Here the antibody fragment targets certain activated platelets, and the payload is successfully delivered to a blood clot. In a control study without the correct antibody no such delivery was achieved, showing that the targeting in the image above was specific in nature. (Work conducted in collaboration with the Baker IDI Institute)

Passive Targeting – Even in the absence of any targeting group, tailoring the size and physico-chemical properties of the dendrimer can achieve preferential accumulation in target tissues or organs (*figure 8*).

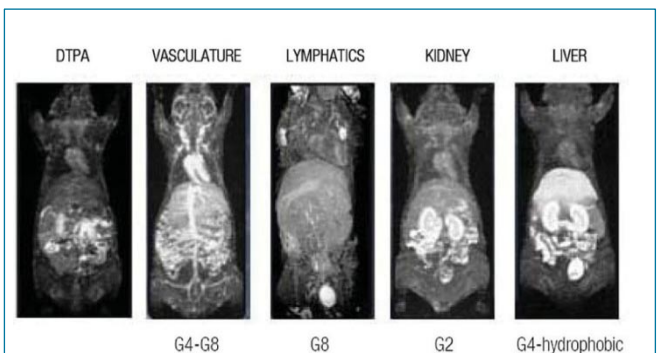
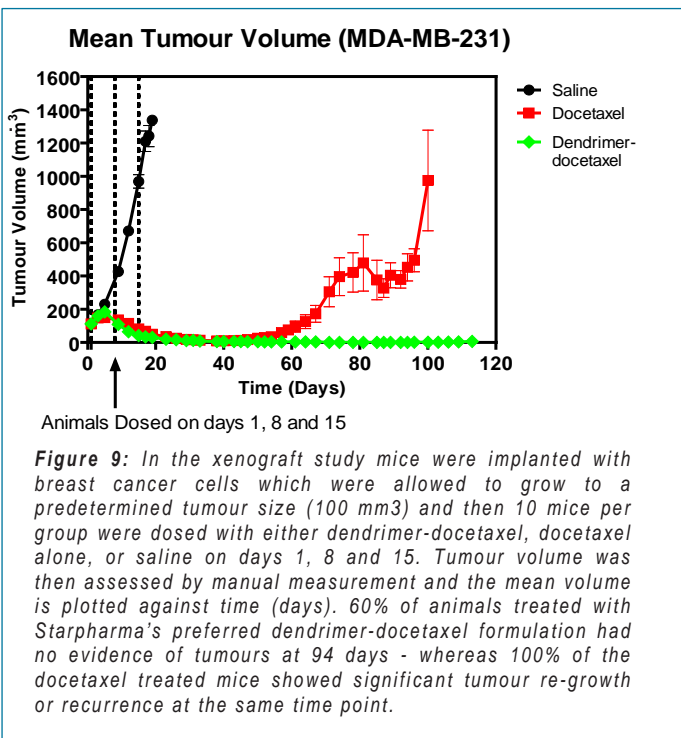


Figure 8: Passive targeting to tissues or organs can still occur in the absence of specific targeting groups. The left image shows the distribution of a non-dendrimer contrast agent control. The other four images are different dendrimer-gadolinium complexes, showing how different dendrimers target different tissues. (Image Courtesy of M Brechbiel, NIH).

IMPROVING EFFICACY

Improved efficacy of the dendrimer-drug formulations is most likely due to a longer circulating half-life, the extended release of drug from the dendrimer and the targeting of the dendrimer construct to diseased tissue. For example Starpharma's dendrimer technology when applied to the leading chemotherapy drug docetaxel resulted in a dendrimer-docetaxel formulation which was significantly more efficacious than docetaxel in a breast cancer model in mice (*figure 9*). Docetaxel is used to treat a wide range of solid tumours including breast, lung and prostate. It is marketed by Sanofi Aventis as Taxotere[®] and generated sales in excess of US\$1.2 billion in 2011.



About Starpharma

Starpharma Holdings Limited (ASX:SPL, OTCQX:SPHRY) is an ASX 300 company and a world leader in the development of dendrimer products for pharmaceutical, life science and other applications. Starpharma's underlying technology is built around dendrimers – a type of synthetic nanoscale polymer that is highly regular in size and structure and well suited to pharmaceutical uses. Starpharma has three core development programs: the VivaGel[®] portfolio, drug delivery, and agrochemicals with the Company developing a number of products internally and others via commercial partnerships. In addition, products for diagnostics and laboratory reagents are already on market through licence arrangements with partners including Siemens Healthcare and Merck KGaA.

Starpharma's lead product is VivaGel[®] (SPL7013 Gel), a gel-based formulation of a proprietary dendrimer. VivaGel[®] is under clinical development for the treatment and prevention of bacterial vaginosis (BV) and also as a vaginal microbicide to prevent the transmission of sexually transmitted infections including HIV and genital herpes. Starpharma has also signed separate licence agreements with Ansell Limited (ASX:ANN) and Okamoto Industries Inc (TSE) to market a value-added, VivaGel[®]-coated condom. Ansell manufactures and sells leading condom brands worldwide, including Lifestyles[®], ZERO[®] and SKYN[®]. Okamoto is the market leader for condoms sold in Japan, the world's second largest condom market.

STRENGTHS OF DENDRIMERS FOR DRUG DELIVERY

- Dendrimer formulations have been shown to significantly enhance the efficacy of the cytotoxic drug docetaxel and will potentially offer similar benefits to other drugs.
- Dendrimer–drug constructs can extend drug half-life and target diseased tissues. Dendrimers can enable substantial improvements in solubility.
- The enhanced solubility achieved through the dendrimer technology has been shown to enable the removal of formulation components responsible for toxic effects in some patients.
- The dendrimer technology can be applied to small molecules, peptides or proteins.
- The payload for small molecule drugs is typically 20-40% w/w.
- A high level of purity / monodispersity can be achieved.
- Dendrimer formulations tend to have relatively low viscosity.
- Dendrimers are synthesised using standard chemical processes and have been manufactured at GMP standards to a multikilogram scale.
- A long shelf life observed in ambient and accelerated stability studies of dendrimer product VivaGel[®] (>2 years ambient).
- Dendrimer constructs often yield a readily soluble powder when freeze dried. Dendrimer breakdown products are primarily natural lysine which is readily processed by the body.
- Dendrimers have a stable covalent structure.
- This contrasts with liposomes which are often considered metastable so that they can eventually rearrange to form planar bi-layers

In the wider pharmaceutical and life science fields, Starpharma has both partnered and internal programs in Drug Delivery including programs with Lilly and GSK. Most recently Starpharma announced pre-clinical results in its docetaxel (Taxotere[®]) program demonstrating significant improvements in that agent's anticancer efficacy and the enhancement of solubility offering potential safety benefits as well. The company is also exploring dendrimer opportunities in agrochemicals in a series of industry partnerships as well as with internal programs including an enhanced version of glyphosate (the active ingredient in Roundup[®]). Starpharma's headquarters and research facilities are located in Melbourne, Australia.

To discuss the potential use of dendrimer technology in your application, please contact:

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